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Modular Recognition of RNA by a Human Pumilio-Homology Domain

X. Wang and T. Hall (NIEHS, NIH), J. McLachlan and P. Zamore (Univ.Mass Med)
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Introduction: Puf proteins are developmental regulators that control mRNA stability and translation by binding sequences in the 3´ untranslated regions (UTRs) of their target mRNAs. All Puf proteins contain a sequence-specific RNA-binding domain comprising eight sequence repeats and N- and C-terminal flanking regions, known as the Pumilio homology domain (PUM-HD) or Puf domain. Crystal structures of the PUM-HDs of *Drosophila* (1) and human (2) PUM proteins revealed that the Puf proteins are alpha-helical repeat proteins with a curved shape, structurally similar to the Armadillo (ARM) repeat proteins, beta-catenin and karyopherin alpha . The RNA-binding specificity of several Puf proteins has been studied. Fly, human, frog, and mouse PUM proteins bind to sequences in the *hunchback* 3´ UTR known as Nanos Response Elements (NREs). UGU triplets are important for RNA binding by Puf proteins generally. Nonetheless, each Puf protein is highly selective, indicating that sequences flanking the UGU core are recognized.

Methods and Materials: The PUM-HD from human Pumilio1 (HsPUM-HD, Gly-828 to Gly-1176) was expressed in *E. coli*, purified as described previously (2). Protein:RNA complexes were crystallized at 20 degrees C by the method of hanging drop vapor diffusion. The structures were determined by molecular replacement using the coordinates of the structure of the HsPUM-HD alone.

Results: We have determined the structure of the HsPUM-HD in complex with NRE RNA. Our studies reveal how this family of RNA-binding proteins interacts with RNA. The structure confirms earlier predictions that the RNA would be bound to the concave surface of the protein. Surprisingly, the rationale for that prediction—that the concave surface of the protein has a net positive charge that could interact with the negatively charged phosphates of the RNA—was wrong; the RNA bases contact the protein while the phosphate groups face the solvent. Although the bases make the primary contacts with the protein, RNA backbone conformation is nonetheless important, since the HsPUM-HD binds RNA >2,500-fold more tightly than the equivalent DNA. The repeated nature of the protein allows recognition of a single RNA base by each of the eight repeats using three amino acid side chains at conserved positions. The structure suggests that RNA recognition is highly modular, and we have confirmed this modularity by designing a simple mutant protein with predictably altered RNA-binding specificity.

Conclusions: The Pumilio-homology domain recognizes RNA on its concave surface in a modular fashion using three side chains from each of its eight repeats to recognize a different RNA base.

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References: (1) T.A. Edwards, S.E. Pyle, R.P. Wharton, and A.K. Aggarwal, “Structure of Pumilio Reveals Similarity between RNA and Peptide Binding Motifs,” Cell*,* **105**, *281-289*.

(2) X. Wang, P.D. Zamore, and T.M.T. Hall, “Crystal Structure of a Pumilio Homology Domain,” Mol Cell, **7**, *855-865*.

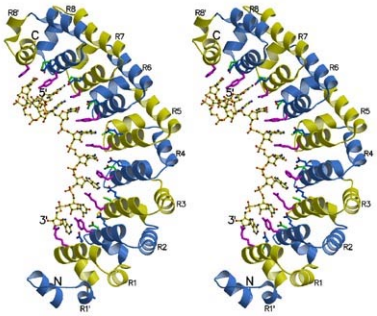

Figure 1 – Ribbon diagram of the HsPUM-HD bound to RNA. The structural repeats are colored alternately blue and yellow. Residues that stack with the RNA bases are magenta, residues that recognize the Watson-Crick face of the bases are blue and green.

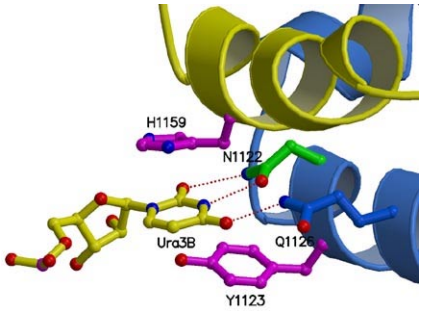

Figure 2 – Recognition of a uracil residue by repeat 8. Tyr-1123 in repeat 8 and His-1159 from repeat 8´ form stacking interactions with the uracil base. Asn-1122 (green) and Gln-1126 (blue) make hydrogen bonds with the uracil base. Hydrogen bonds are indicated with red dotted lines.